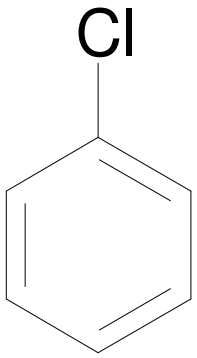
CHLOROBENZENE

(CAS Reg. No. 108-90-7)



INTERIM ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

For

NAS/COT Subcommittee for AEGLS

2009

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicological and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. Three levels X AEGL-1, AEGL-2 and AEGL-3 X are developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m;) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects, or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m;) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

TABLE OF CONTENTS

LIST OF TABLES

EXECUTIVE SUMMARY

Chlorobenzene is a flammable liquid with a high vapor pressure and a medium solubility in water. The product is used as a solvent and for the production of nitrochlorobenzene and intermediates for the synthesis of dyestuffs, pharmaceuticals and products for the rubber and plastic industry. Chlorobenzene has an aromatic, almond-like odor. The odor threshold in water is 0.050 mg/L (Verschueren 1983 as quoted by ATSDR 1990). The odor threshold in air is reported to be 0.2 - 1.8 ppm, although also a value 62 ppm has been reported.

The database for chlorobenzene is rather poor and many data are limitedly described in reviews or summaries; several original papers were unavailable. Human data are limited to two kinetic studies with volunteers. Animal data are available on teratogenicity and reproduction toxicity and on mortality. A few studies with experimental animals addressing CNS depression were available but difficult to interpret.

The AEGL-1 values are based on two kinetic studies with volunteers. Ogata *et al.* (1991) exposed four male volunteers to chlorobenzene. They were exposed to 60 ppm for 7 hours with a 1-hour break after 3 hours, and were asked to report on some subjective complaints. All volunteers complained of a sensation of disagreeable odor and of drowsiness. Three out of four had a heavy feeling in the head and/or headache, two out of four a throbbing pain in the eyes and one out of four a sore throat. No information was given about the time of onset of these complaints. Flicker fusion frequency values were significantly decreased from 39.1 to 35.9 cycles/s at the end of the 3-hour exposure period. No further effect was seen in the afternoon. Knecht and Woitowitz (2000) exposed eight volunteers to 10 ppm for 8 hours a day for 5 days. None of the volunteers made complaints with respect to chemical-irritative effects as irritation of eyes and the nasal region, respiratory tract or skin, allergic or dermal reactions or acute central nervous system depressant with signs of headache, dizziness or fatigue. The effects reported by Ogata *et al.* (1991) are considered to be indicative of discomfort. Therefore, the 10 ppm (8-hour) exposure concentration was used as a conservative point of departure. Because human data are used, the interspecies uncertainty factor is 1. Since the effects observed at 60 ppm were considered to be mild some safety margin is already present when using 10 ppm as point of departure. Therefore an intraspecies factor of 1 was considered sufficient. This results in an 8-hour AEGL-1 value of 10 ppm. Since the described effects at 60 ppm include irritation and CNS depression, the 8-hour AEGL-1 value of 10 ppm is considered appropriate for all time-points. This is supported by the fact that Knecht and Woitowitz (2000) reported that the chlorobenzene levels in blood reached a steady-state level within one hour.

There are no adequate human data for derivation of AEGL-2. Some studies with experimental animals report subtle CNS effects but are difficult to interpret with respect to their relevance for humans and for AEGL-2 derivation. The effects reported by Frantik *et al.* (1994) and De Ceaurriz *et al.* (1993) are considered to represent sub AEGL-2 effects. The most appropriate point of departure is the absence of AEGL-2 related effects in rats and guinea pigs exposed to 2990 ppm for 30 min (UBTL, 1978). An interspecies UF of 3 is considered sufficient because 1) data were comparable for rats and guinea pigs suggesting no large interspecies differences and 2) the critical effect is CNS depression. Further, the use of a larger interspecies UF (next to an intraspecies UF of 3) would result in AEGL-2 levels that conflict with human data (Ogata *et al*., 1991). Therefore, an intraspecies UF of 3 is chosen resulting in an overall UF of 10 and a 30-min AEGL-2 value of 300 ppm. The value of 300 ppm for 30 min was extrapolated across time periods using $C^n \times t = k$ with default values $n = 1$ for extrapolation to 1 hour and $n=3$ for extrapolation to 10 min. The 4- and 8-hour AEGL-2 values were set equal to the 1-hour value because 1) chlorobenzene concentrations in blood reach a steady-state within 1 hour and elimination is rapid and 2) time scaling with $n = 1$ would result in 4- and 8-hour AEGL-2 values (37 and 19 ppm, respectively) that conflict with human data (Ogata *et al*. 1991).

Several mortality studies were reported; however most of these studies were only available as a summary and could not be judged on their merits. Bonnet *et al.* (1979; 1982) reported a 6-hour LC_{50} of 2965 ppm for male rats and a 6-hour LC_{50} of 1886 ppm for mice. No deaths were reported in rats and guinea pigs (5 animals per sex per species) exposed to chlorobenzene concentrations of up to 7970 ppm for 30 min (UBTL, 1978). The data in rats and guinea pigs reported by UBTL (1978) are considered to provide the most appropriate point of departure for AEGL-3 derivation, i.e. no mortality in rats and guinea pigs after exposure to 7970 ppm for 30 min. The rationale for the choice of an interspecies and intraspecies UF (of 3 each) and for time scaling (using default values for n for extrapolation to 10- and 60-min and flatlining to 4- and 8-hours) is similar to that for AEGL-2. These values are considered to be consistent with the AEGL-2 values and are supported by the 6-hour LC_{01} of 1873 ppm calculated from the probit equation reported by Bonnet *et al*. (1982).

Summary of AEGL Values for Chlorobenzene

Classification	10-minute	30-minute	1-hour	4-hour	8-hour	Endpoint (Reference)
AEGL–1[a] (Nondisabling)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	Highest level without AEGL-1 effects in humans (Ogata *et al.*, 1991; Knecht and Woitowitz, 2000)
AEGL–2 (Disabling)	430 ppm (2021 mg/m^3)	300 ppm (1410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	Narcosis (UBTL, 1978)
AEGL–3 (Lethal)	1100 ppm (5170 mg/m^3)	800 ppm (3760 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)	No mortality in rats and guinea pigs (UBTL, 1978)

References

Bonnet, P., Raoult, G., Gradiski, D. 1979. Concentrations léthales 50 des principaux hydrocarbures aromatiques. Arch. Mal. Prof. 40:805-810.

Knecht, U., Woitowitz, H.J. 2000. Human toxicokinetics of inhaled monochlorobenzene: Latest experimental findings regarding re-evaluation of the biological tolerance value. Int. Arch. Occup. Environ. Health 73:543-554.

Ogata, M., Taguchi, T., Hirota, N., Shimada, Y., Nakae, S. 1991. Quantitation of urinary chlorobenzene metabolites by HPLC: Concentrations of 4-chlorocatechol and chlorophenols in urine and of chlorobenzene in biological specimens of subjects exposed to chlorobenzene. Int. Arch. Occup. Environ. Health 63:121-128.

UBTL 1978. Utah Biomedical Test Laboratory Report on NIOSH sponsored inhalation study for IDLH values (Final report). With cover letter dated 102291. U.S. EPA/OPTS Public Files OTS0534605.

1. INTRODUCTION

Chlorobenzene is a flammable liquid with a high vapor pressure and a medium solubility in water. No information on current production volumes was available. This substance is commercially produced by the chlorination of benzene in the presence of a catalyst (ATSDR 1990).In 1992, the production volume of chlorobenzene was 231 million pounds in the US (US EPA, 1995). The product is used as a solvent and for the production of nitrochlorobenzene and intermediates for the synthesis of dyestuffs, pharmaceuticals and products for the rubber and plastic industry (BUA 1990).

Chlorobenzene has an aromatic, almond-like odor. The odor threshold in water is 0.050 mg/L (Verschueren 1983 as quoted by ATSDR 1990). The odor threshold in air is 0.2 - 1.8 ppm (Verschueren 1983 as quoted by ATSDR 1990). A low odor threshold of 0.2 ppm was reported by Ruth (1986) in an overview. Also a high odor threshold of 62 ppm was stated.

Table 1. Chemical and Physical Properties

Parameter	Value	Reference
Synonyms	monochlorobenzene; benzene chloride; phenylchloride; MCB; chlorobenzol	
Chemical formula	C_6H_5Cl	
Molecular weight	112.56	
CAS Reg. No.	108-90-7	
Physical state	liquid	ATSDR 1990
Color	colorless	ATSDR 1990
Solubility in water	500 mg/L (20°C)	ATSDR 1990
Vapor pressure	8.8 mm Hg (20°C)	ATSDR 1990
Vapor density (air = 1)		
Liquid density (water = 1)	1.1058 g/cm^3	ATSDR 1990
Melting point	-45.6°C	ATSDR 1990
Boiling point	132 °C	ATSDR 1990
Odor	aromatic, almond-like	ATSDR 1990
Flammability	1.8%-9.6%	ATSDR 1990
Explosive	LEL 1.3%	NIOSH pocket guide to Chemhaz
Conversion factors	1 mg/m^3 = 0.22 ppm 1 ppm = 4.7 mg/m^3	ATSDR 1990

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

No data available.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

Several reviews including ACGIH (1991) and Hellman (1993) cited reports in which the effects of inhalation and oral ingestion of chlorobenzene are described as drowsiness, incoordination, and

unconsciousness as well as irritation of the eyes and respiratory tract. However, indications of exposure levels were not provided.

An irritation concentration of 205 ppm was reported by Ruth (1986) in an overview, but the source of this information was not given.

2.2.2. Experimental Studies

Four adult male volunteers, exposed to airborne chlorobenzene as part of an investigation into the excretion of urinary metabolites were asked to report on some subjective complaints (Ogata *et al.* 1991). The volunteers were exposed in a room to 60.2 ± 3.9 ppm for 3 h in the morning and 4 h in the afternoon with a 1 h break in between. The concentrations were determined by gas chromatography and were reported to be constant within a 5% range. All volunteers complained of disagreeable odor and drowsiness. Three out of four had a heavy feeling in the head and/or headache, two out of four a throbbing pain in the eyes and one out of four a sore throat. No information was given about the time of onset of these complaints. Chlorobenzene exposure did not affect pulse rates or systolic and diastolic pressure. Flicker fusion frequency values (frequency at which successive flashes are seen as continuous) were reduced significantly from 39.1 to 35.9 cycles/s at the end of the 3-hour exposure period in the morning. No further effect was seen in the afternoon. The significance of this finding is difficult to interpret.

Eight volunteers were exposed to 10 ppm for 8 hours a day for 5 consecutive days to determine the relation between chlorobenzene exposure and urine levels of the chlorobenzene metabolites 4-chlorocatechol and chlorophenols (Knecht and Woitowitz 2000). None of the subjects made a complaint. This applied to chemical-irritative effects as irritation of eyes and the nasal region, respiratory tract or skin, allergic or dermal reactions or acute central nervous system depressant with signs of headache, dizziness or fatigue (Knecht: personal communication, 2005).

2.2.3. Occupational / Epidemiological Studies

The consequences of occupational exposure to chlorobenzene are described in the IRPTC (1988) summary on chlorobenzenes. These cases are not included here because the concentration was unclear and possible co-exposure to other chemicals was unknown.

2.3. Neurotoxicity

In the IRPTC summary (1988) individual EEG changes were described both at the time of inhaling and as near- and long-term effects. The chlorobenzene concentration of 0.2 mg/m^3 (0.044 ppm) appeared as the threshold level (exposure period unknown) by the criterion of changes in electrical brain activity, with 0.1 mg/m^3 (0.022 ppm) as the no-effect concentration. No further details were provided in the IRPTC summary and the original publications were not available. Therefore, these results are considered only as supplementary information.

2.4. Summary of human data

No information is available concerning the acute lethal concentrations or duration of exposure in humans. Available information shows that chlorobenzene in air can be irritating to the eyes and the respiratory tract with signs of CNS depression after 7 hours of exposure to 60 ppm. It is noted that odor might have interfered with the reportage of subjective complaints of irritation. No complaints of irritation were described in another volunteer study in which humans were exposed to 10 ppm for 8 h/d for 5 days.

3. ANIMAL TOXICITY DATA

3.1. Acute lethality

3.1.1. Cats

Gotzmann (1904, as cited in Flury and Zernik (1931)) studied the effect of inhalation of chlorobenzene to cats. Only limited information is available stating that exposure to 220 to 660 ppm was bearable for hours, 1200 ppm for an unknown period induced clear narcosis, 2400 - 2900 ppm induced unsteady movement, tremors and convulsions within one hour but no more severe damage; 3700 ppm caused mortality and pulmonary hemorrhage was seen after 7 hours; 8000 ppm for 2 hours induced signs of narcosis after 30 minutes and mortality after the end of the exposure period. Considering the limited available information, this study can only be considered as supporting information.

3.1.2. Rabbits

Respiratory exposure of rabbits (head only or whole body, sex and number of animals unknown) to chlorobenzene for four hours at concentrations of 550 ppm to 660 ppm induced lethality after two weeks, whereas no visible changes in test animals were observed at concentrations of 110 to 220 ppm (Rozenbaum 1947 as cited by IRPTC 1988). Probably referring to the same study, the ATSDR (1990) stated that rabbits died 2 weeks after exposure to concentrations of about 537 ppm for 2 hours (Rozenbaum 1947 as cited by ATSDR 1990). The original 1947 publication could not be retrieved, but these results are in contrast to the findings in other studies, e.g. the absence of mortality in the range finding and main study on developmental toxicity in rabbits (Chapter 3.4).

Repeated exposure of 32 male rabbits to 248 ppm for up to 24 weeks failed to increase mortality (Dilley, 1977). In addition, no mortality was observed in a teratogenicity study (1000 ppm, 6 h/d for 10 days), but deaths were observed at 3000 ppm (John *et al.*, 1984).

3.1.3. Guinea pigs

Groups of 5 guinea pigs per sex were whole body exposed to mean analytical concentrations of 2990 (SD: 53), 5850 (1350) or 7970 (355) ppm chlorobenzene for 30 min and held under observation for 14 days. No deaths were observed at any concentration (UTBL, 1978).

3.1.4. Rats

Bonnet *et al.* (1982) determined the 6-hour LC_{50} of chlorobenzene in male rats (Sprague-Dawley). Twelve rats per concentration were used and observed for 14 days after total body exposure. Nominal test concentrations were not provided. Actual concentrations were determined using gas chromatography, but information on the exposure concentrations were limited to a graph on log scale. It can be estimated from this graph that the lowest concentration tested in rats was approximately 2000 ppm and this exposure was associated with 8% mortality. The LC_{50} was 2965 ppm (95% CI: 2787 - 3169 ppm) with a regression line of *Probit = -33 + 10.9 logC*. (It is noted that Bonnet *et al*. presented a positive intercept (i.e. **+**33) but it was concluded from the data that this should be **-**33). The following effects were observed during exposure: hypotony, stereotypy, somnolence, tremor and muscle contractions.

An LC_{100} value of 4400 ppm in rats after a 2-hour exposure was reported by Rozenbaum (1947, as cited by BUA 1990). However, according to ATSDR (1990), this study was performed in mice. The original publication could not be retrieved.

In the Eastman Kodak Company review from 1994 to the EPA (Anonymous, 1994), the authors stated stated “Acute exposure to 22,000 ppm for 3½ hours killed 2/3 rats in 2½ hours while 9000 ppm for

6 hours killed 2/3 rats in 3 hours" with reference to unpublished data of Eastman Kodak Company. The original study is not available.

Groups of 5 rats per sex were whole body exposed to mean analytical concentrations of 2990 (SD: 53), 5850 (1350) or 7970 (355) ppm chlorobenzene for 30 min and held under observation for 14 days. No deaths were observed at any concentration (UBTL, 1978).

Repeated exposure of 32 male rats to 248 ppm for up to 24 weeks did not result in mortality (Dilley, 1977). In addition, no mortality was observed in a 2-generation study (450 ppm, 6h/d; 7d/w for 17 weeks) (Nair *et al.*, 1984) or in a rat developmental toxicity study (1000 ppm, 6 h/d for 10 days (John *et al.*, 1984). However, in the latter study increased mortality was observed at 3000 ppm (John *et al.*, 1984).

3.1.5. Mice

Bonnet *et al.* (1979) determined the 6-hour LC_{50} of monochlorobenzene in female mice (OF_1). Twenty-five mice per concentration were used and observed for 14 days after total body exposure. Nominal test concentrations were not provided. Actual concentrations were determined using gas chromatography. The analytical concentrations were between 90 and 100% of the nominal concentrations. No details on the exposure concentrations used were provided but a graph on log scale. It can be estimated from the concentration-response graph that the lowest dose tested in mice was approximately 1500 ppm and caused approximately 20% mortality. The LC_{50} was 1886 ppm (95% CI: 1781 - 1980 ppm) with a regression line of *probit = -17.06 + 6.734 logC*. (It is noted that Bonnet *et al*. presented a positive intercept (i.e. **+**17.06) but it was concluded from the data that this should be **-**17.06).

An LC_{50} of 4070 ppm was reported in the mouse for a two hour exposure in the IRPTC summary on chlorobenzenes (Sanotsky and Ulanova 1975 as cited by IRPTC 1988). The LC_{16} and LC_{84} were 2244 ppm and 7832 ppm, respectively. The original 1975 study could not be retrieved. Also according to the IRPTC summary, exposure for an unknown duration to 2200 ppm failed to kill mice but 4400 ppm killed 3 out of 4 mice (Gizhlaryan (1961) as cited by IRPTC (1988)). This study could not be retrieved.

An LC_{100} value of 4400 ppm after exposure for 2 hours was reported by Rozenbaum (1947, as cited by ATSDR 1990). However, according to BUA (1990), this study was performed in rats. Again, the original publication could not be retrieved.

TABLE 2. Summary of Selected Relevant Acute Lethal Inhalation Data in Laboratory Animals

Species	Concentration (ppm)	Exposure Time	Effect[a]	Reference
Rats (male)	2965	6 hours	LC_{50}	Bonnet *et al*. 1982
Rats (or mice)	4400	2 hours	LC_{100}	Rozenbaum 1947 as cited by BUA 1990
Cats	3700	7 hours	Mortality	Flury and Zernik 1931
Cats	8000	2 hours	Mortality	Flury and Zernik 1931

TABLE 2. Summary of Selected Relevant Acute Lethal Inhalation Data in Laboratory Animals

Guinea pigs	7970	30 min	No mortality	UBTL, 1978
Rats	22000	3.5 hours	Mortality in 2 out of 3	Anonymous, 1994
Rats	9000	6 hours	Mortality in 2 out of 3	Anonymous, 1994
Rats	7970	30 min	No mortality	UBTL, 1978
Mice (female)	1886	6 hours	LC_{50}	Bonnet *et al*. 1979
Mice	7832 4070 2244	2 hours 2 hours 2 hours	LC_{84} LC_{50} LC_{16}	Sanotsky and Ulanova 1975, as cited by IRPTC 1988
Repeated exposures				
Rat (2-generation study)	450	6 hours/day for 7 days/week up to 17 weeks	No mortality	Nair *et al*. 1984
Rabbits (pregnant)	3000 1000	6 hours/day for 13 days	Mortality No mortality	John *et al*. 1984
Rats (pregnant)	3000 1000	6 hours/day for 10 days	Mortality No mortality	John *et al*. 1984
Rats	248	7h/d; 5 d/w for 24 w	No mortality	Dilley 1977

3.2. Nonlethal toxicity

3.2.1. Cats

Gotzmann (1904, as cited in Flury and Zernik 1931) studied inhalation exposure of chlorobenzene in cats. Only limited information is available stating that exposure to 220 to 660 ppm was bearable for hours and exposure to 1200 ppm for an unknown period induced clear signs of narcosis; cats that inhaled 2400 - 2900 ppm developed unsteady movement, tremors and convulsions within one hour but no severe damage. Exposure to 3700 ppm caused mortality and lung hemorrhages after 7 hours and exposure to 8000 ppm for 2 hours produced narcosis effect after 30 minutes and mortality increased after the end of the exposure. Considering the limited available information, this study can only be used as additional information.

3.2.2. Guinea pigs

Groups of 5 guinea pigs per sex were whole body exposed to mean analytical concentrations of 2990 (SD: 53), 5850 (1350) or 7970 (355) ppm chlorobenzene for 30 min and held under observation for 14 days. No deaths were observed at any concentration. At 2990 ppm slight eye and nasal irritation was

observed but none of the animals was judged to suffer from impaired ability to escape. At the next higher concentration, 5850 ppm, all guinea pigs suffered from narcosis and were judged to have impaired ability to escape. No deaths occurred at the highest concentration but ataxia occurred within 10 min and narcosis was evident after 15 min (UBTL, 1978).

3.2.3. Rats

Groups of 5 rats per sex were whole body exposed to mean analytical concentrations of 2990 (SD: 53), 5850 (1350) or 7970 (355) ppm chlorobenzene for 30 min and held under observation for 14 days. No deaths were observed at any concentration. At 2990 ppm slight eye and nasal irritation was observed but none of the animals was judged to suffer from impaired ability to escape. At the next higher concentration, 5850 ppm, most rats suffered from narcosis and were judged to have impaired ability to escape; a quick recovery after exposure was reported. No deaths occurred at the highest concentration but ataxia was present at 10 min and narcosis was evident in all animals after 25 min of exposure (UBTL, 1978).

Frantik *et al*. (1994) investigated the relative neurotoxicity of a large series of solvents. Rats (male adult albino SPF, n=4 per group) were exposed to at least three concentrations of chlorobenzene (analytical purity) or ambient air. Inhalation was performed in a dynamic system for 4h and concentrations were measured by GC. The actual exposure concentrations used were not provided. Most animals were used three or four times with intervals of 3 weeks. Directly after an inhalation period the animals received a short electrical pulse through ear electrodes. In rats, the duration of subsequent tonic extension of the hind limbs was determined. This parameter was shown to be the most sensitive and consistent. The study authors calculated the concentration required to induce a 37.5% change in the neurological response, i.e. decrease in duration of the toxic extension from 8 to 5 seconds. For chlorobenzene, a 37.5%-effect concentration of 611 ppm was reported (90% confidence interval of 538-684 ppm). The slope was 0.061 %/ppm. This 37.5% response level corresponds, according to the study authors, to a concentration that does not influence normal locomotor activity or induce behavioral excitation (e.g. for the aromatics). Hence, this 37.5% effect level is a quite sensitive neurological endpoint.

Rebert *et al.* (1995) studied the effect of inhalation exposure of male rats to chlorobenzene on the auditory system. Groups of 8 or 9 male Long Evans rats were exposed whole body for 8 hours a day for 5 days to target concentrations in the range of 500 to 2400 ppm. The analytical concentrations were within 10% of the target concentrations determined using gas-chromatography. Auditory function was determined between 3 and 13 days after the end of exposure using the brainstem auditory-evoked response (integrated amplitude) elicited by 16 kHz tone pips over a range of 25 - 95 dB with 10 dB increments. The average response over 55 through 85 dB was compared to controls. A reduction in body weight gain was observed with exposure to 2000 and 2400 ppm. No information was available on body weights of animals that inhaled 1500 ppm or less. A reduction in the integrated amplitude of the response was found after exposure to 2000 ppm or 2400 ppm and approximately 1500 and 2000 ppm (other experiment, estimated from figure) but not at approximately 500 and 1000 ppm (estimated from figure). For one of the experiments with chlorobenzene the effect was still present at 4 weeks after exposure. Although it was not a subject in the study by Rebert *et al.,* it is known for other organic solvents that exposure can result in permanent hearing loss due to the destruction of cochlear hair cells. Other effects are not described in this study. It is noted that the highest tested concentration of 2400 ppm is close to the 6-hour LC_{50} of 2965 ppm in the rat study by Bonnet *et al.* (1982).

3.2.4. Mice

Frantik *et al.* (1994) investigated the relative neurotoxicity of a large series of solvents. Mice (female H strain, n=8 per group) were exposed to at least three concentrations of chlorobenzene (analytical purity) or ambient air. Inhalation was performed in a dynamic system for 2h and concentrations were measured by GC. The concentrations of chlorobenzene used were not defined. Most animals were used three or four times with intervals of 3 weeks. Immediately after inhalation, the animals received a short electrical pulse through ear electrodes. The velocity of tonic extension due to toxicity was determined. This parameter was shown to be the most sensitive and consistent. The authors calculated the concentration needed to induce a 30% change in the neurological response, i.e. decrease in velocity of the tonic extension. For chlorobenzene, a 30%-effect dose of 610 ppm was reported (90% confidence interval of 320-900 ppm). The slope was 0.041%/ppm. This 30% response level corresponds, according to the study authors, to a concentration that does not influence normal locomotor activity or induced behavioral excitation.

De Ceaurriz *et al.* (1983) tested the effect of chlorobenzene inhalation on the duration of immobility during a 3-minute "behavioral despair" swimming test. Groups of 10 male Swiss OF_1 mice were exposed (whole body) for four hours to 0, 650, 785, 875 or 1000 ppm chlorobenzene. The analytical concentrations were determined using gas-liquid chromatography. However, the analytical results were not provided and it must be assumed that the stated concentrations are the analytical concentrations. At the end of exposure the mice were placed in water and the duration of immobility during a 3-minute period was determined and compared to the control animals. A significant and dose-dependent decrease in immobility of -28%, -45%, -53% and -82% was found at respectively 650, 785, 875 or 1000 ppm. The ID_{50} was determined at 804 ppm (95% CI: 718-887 ppm).

De Ceaurriz *et al.* (1981) determined the RD_{50} for sensory irritation of monochlorobenzene in mice. Groups of 6 male Swiss OF_1 mice were exposed head only for 5 minutes to at least 4 different concentrations of monochlorobenzene. The respiratory rate was determined during exposure with a plethysmograph. The analytical concentration was determined using gas-chromatography but it is unclear whether the stated RD_{50} was based on target, nominal or analytical concentrations. The RD_{50} was calculated as 1054 ppm.

Aranyi *et al.* (1986) examined the effect of inhalation exposure of chemical air contaminants including chlorobenzene on murine host defenses. Groups of approximately 150 female mice from 5 replicates were exposed once for three hours or five times on five consecutive days to 75 ppm chlorobenzene. The analytical concentrations were determined using gas-chromatography and were in close agreement with the target concentrations and host defence status was determined by simultaneous exposure to viable *Streptococcus zooepidemicus*. Ensuing deaths were recorded daily over a 14-day observation period. Exposure to 75 ppm chlorobenzene failed to influence either the mortality from streptococcus challenge or bactericidal activity.

TABLE 3. Summary of Selected Acute Nonlethal Inhalation Data in Laboratory Animals

Species	Concentration (ppm)	Exposure Time	Effect	Reference
Guinea pigs	2990	30 min	Slight eye and nasal irritation; no impaired ability to escape	UBTL, 1978
	5850	30 min	Narcosis in all guinea pigs	
	7970	30 min	Ataxia within 10 min and narcosis within 15 min	

TABLE 3. Summary of Selected Acute Nonlethal Inhalation Data in Laboratory Animals

Rats	2990	30 min	Slight eye and nasal irritation; no impaired ability to escape	UBTL, 1978
	5850	30 min	Narcosis in most rats	
	7970	30 min	Ataxia at 10 min and narcosis within 25 min	
Rats (male)	1500	8 hours/day for 5 days	Reduction in auditary-evoked respons	Frantik *et al.* 1994
	1000		No effect	
Rats (male)	611	4 hours	Shortening of the tonic extension of the hind limbs by 37.5% after electrical stimulation	Frantik *et al.* 1994
Cats	8000	½ hour	Narcotic	Gotzmann 1904 as cited by Flury and Zernik 1931
		2 hours	Mortality	
	2400-2900	1 hour	Unsteady movement, tremors and convulsions	
	1200	unknown	Clear narcotic effects	
	220-660	several hours	Bearable for hours	
Mice (male)	1054	5 minutes	RD_{50} for sensory irritation	De Ceaurriz *et al*. 1981
mice	75	3 hours once or on 5 days	No effect on murine host defense	Aranyi *et al.* 1986
Mice (female)	610	2 hours	Increase in velocity of the tonic extension of the hind limbs by 30% after electrical stimulation	Frantik *et al.* 1994
Mice (male)	650	4 hours	Decrease in immobility in the "behavioral despair" swimming test by 2	De Ceaurriz *et al*. 1983

3.3. Developmental / Reproductive toxicity

Groups of 32 to 33 pregnant female F344 rats were exposed by inhalation (whole body) for 6 hours a day to 0, 75, 210 or 590 ppm (nominal concentration) chlorobenzene (purity 99.982%) from day 6 through 15 of gestation (John *et al*. 1984; Hayes *et al.* 1981). These exposure conditions were chosen on the basis of a preliminary study in which test atmospheres of 0, 300, 1000, and 3000 ppm were generated and 10 rats per concentration were exposed 6 hours/day on gestation days 6 through 15 and sacrificed on day 16. Test animals were killed on day 21 of gestation and the fetuses examined. The chlorobenzene concentration in the chamber was determined with infrared spectrophotometry. The time-weighted average analytical concentrations were within 7 to 8% of the targeted concentrations.

In the range-finding study exposure to 3000 ppm induced severe irritation of the eyes and nasal area, signs of narcosis, and mortality (or sacrificed in a moribund state) at an unstated time during the exposure period. The effects observed at 1000 ppm included a reduction in absolute body weight, reduced

food-consumption, internal and external lesions, an increase in relative kidney and liver weights, reduction in thymus size and an increase in the number of resorptions. At 300 ppm, only a small decrease in body weight gain (day 6-8) and an increase in relative liver weight were observed.

In the main study, maternal toxicity was restricted to the highest dose group (590 ppm) and consisted of a significant reduction in weight gain on day 6-8 and a significant increase in absolute and relative liver weights. No effects were found on pregnancy rate, litter size, resorptions, fetal bodyweights or the incidence of external or soft tissue alterations. At the highest dose some increases in skeletal variations such as a delay in ossification were found (John *et al*. 1984, Hayes *et al.* 1981).

In a second study, groups of 30 pregnant female NZW rabbits were exposed by inhalation (whole body) for 6 hours/day to 0, 75, 210 or 590 ppm (nominal concentration) chlorobenzene (purity 99.982%) from day 6 through 18 of gestation (John *et al*. 1984, Hayes *et al.* 1981). These exposure conditions were chosen on the basis of a preliminary study in which test atmospheres of 0, 300, 1000 and 3000 ppm were tested on 7 rabbits per concentration during 6 hours/day on day 6 through 18 and sacrificed on day 19. Test animals were killed on day 29 of gestation and the fetuses examined. The chlorobenzene concentration in the chamber was determined with infrared spectrophotometry. The time-weighted average concentration was within 7 to 8% of the targeted concentrations.

The effects observed in the range-finding at 3000 ppm were mortality (or sacrificed in a moribund state) during exposure, severe systemic and hepatotoxicity, reduced weight gain and macroscopic changes of the liver. The effects observed at 1000 ppm included reduced bodyweight gain on day 6-8 and macroscopic changes of the liver. Slight liver effects (not described) were found at 300 ppm.

Maternal toxicity was restricted to the 210 and 590 ppm exposure groups and consisted of a significant increase in absolute and relative liver weights. No effects were found on pregnancy rate, litter size, resorptions, and fetal body weights. A low and non-significant increase in head and facial anomalies and heart defects was found in the 210 and 590 ppm dose groups but not in the concurrent controls. The incidence of an extra (thoracic) rib (variation) was significantly increased in the offspring from does inhaling 580 ppm (John *et al*. 1984; Hayes *et al.* 1981).

Because of the low increase in malformations the rabbit study was repeated using the same conditions and concentrations of 10, 30, 75 and 590 ppm. Maternal toxicity was restricted to the 590 ppm dose group and consisted of an increase in liver weight. The percentage of litters with resorptions was significantly increased at 590 ppm, but this observation was within the historical control range. No effects were found on pregnancy rate, litter size, fetal body weights or the incidence of external, skeletal or soft tissue alterations (John *et al*. 1984, Hayes *et al.* 1981).

It is concluded from this study that chlorobenzene does not induce teratogenic or embryolethal effects in rats and rabbits when tested up to maternally toxic levels. Chlorobenzene is not considered to be a developmental toxicant.

Nair *et al.* (1987) performed a 2-generation reproduction study with chlorobenzene in rats exposed by inhalation. Groups of 30 male and 30 female CD rats were exposed whole body to target concentrations of 0, 50, 150 or 450 ppm chlorobenzene for 6 hours/day, 7 days per week. The analytical concentration was determined using a MIRAN 1A organic vapor analyzer. The analytical concentrations were comparable to the target concentration and were 10% above the nominal concentration. No effects were seen on mortality, body weight, food consumption, reproductive parameters, pup viability and survival. Liver toxicity was mainly seen at 150 and 450 ppm. Increases in the incidence of small flaccid testis and in incidence (6 out of 30) and severity of unilateral or bilateral degeneration (minimal to severe) of the germinal epithelium were found at 450 ppm in both generations. Three of six affected males at the highest dose in each generation sired litters. However, this effect was not reflected in the overall fertility

of the high dose males. Small increases of these effects were also seen at 150 ppm. An increase in the incidence of dilated renal pelvis was observed in high dose males of the F0 generation and in all treated male groups of the F1 generation. Microscopically, an increase in renal degeneration and inflammatory lesions was found at the two highest concentrations.

The transfer of chlorobenzene to the fetus of pregnant mice after inhalation exposure to 500 ppm for 1 hour was shown by Shimada (1988b, in Japanese).

It can be concluded that chlorobenzene does not affect the fertility in rats when tested up to exposures that induced overt maternally toxicity.

3.4. Genotoxicity

The genotoxic potential of chlorobenzene has been summarized (IPCS, 1991; NTP, 1985; ATSDR, 1990; BUA, 1990). The conclusions of these summaries are described below and additional studies published afterwards are added.

In vitro studies

Chlorobenzene was negative when tested in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without, the addition of an S9 fraction from the liver of Aroclor 1254-treated rats (Haworth *et al*. 1983 as cited by IPCS 1991) (NTP, 1985). Chlorobenzene was also negative when tested in the TA1538 strain (Shimizu *et al*. 1983 as cited by IPCS 1991). Lawlor *et al.* (1979 as cited by NTP 1985) reported that chlorobenzene was negative in TA98, TA100, TA1535, TA1537 or TA1538 when tested with and without S9, in *E. coli* strains WP2 uvr A+ rec A+ or WP100 uvr A- rec A-, TA1978 uvr B+ or TA1538 uvr B-. Chlorobenzene induced a dose-dependent increase in revertants in *Actinomycetes antibioticus*-400 (Keskinova 1968 as cited by NTP 1985). Negative results were obtained in a gene mutation assay in *Aspergillus nidulans* without activation (Prasad 1970 as cited by ATSDR 1990) (Prasad and Pramer 1968 as cited by BUA 1990). Further, chlorobenzene was also negative in TA100, TA1535, TA92, TA1537, TA98 and TA1538, with or without activation, in a study by Simmon (1984 as cited by BUA 1990). A gene mutation test in *Saccharomyces cerevisiae*, with and without activation also revealed negative results (Monsanto Company 1976 as cited in BUA 1990). Chlorobenzene was found positive in a gene mutation test in *Saccharomyces cerevisiae*, with and without activation (Simmon *et al.* 1979 as cited by BUA 1990).

Chlorobenzene was negative in two DNA repair test in rat cells (UDS) *in vitro* (Shimada *et al.,* 1983 as cited by ATSDR, 1990) (Williams *et al.,* 1989 as cited by BUA, 1990), as well as in a gene mutation test in mouse lymphoma L5178Y with and without activation (Monsanto Company, 1976 as cited by BUA, 1990). The substance was positive in a gene mutation test in mouse lymphoma L5178 tk+/tk- with and without activation (McGregor *et al.,* 1988 as cited by BUA, 1990), and in an *in vitro* sister chromatid exchange test without metabolic activation but negative with metabolic activation (Loveday *et al.,* 1989 as cited by BUA, 1990). Negative results were obtained in an *in vitro* chromosome aberration test with and without activation (Loveday *et al.,* 1989 as cited by BUA , 1990). Exposure of rat bone marrow cells to chlorobenzene resulted in a dose-dependent decrease in cell proliferation and mitotic indices and a dose-dependent increase in sister chromatid exchange (Khalil and Odeh, 1994).

The provided *in vitro* studies indicate that chlorobenzene does not induce DNA damage or gene mutations in bacterial tests. Contradicting results were found in gene mutation tests on yeasts and DNA damage and gene mutation tests on mammalian cells. The only *in vitro* test on chromosome mutations was negative. Based on the *in vitro* tests, the possibility of DNA damage and gene mutations after chlorobenzene exposure cannot be excluded.

In vivo studies on animals

Chlorobenzene binds covalently to DNA in the mouse liver, kidney and lung and rat tissue (Grilli *et al*., 1985 as cited by IPCS, 1991). According to BUA (1990) this might be due to contamination of the DNA with RNA or proteins. A guanine DNA adduct was found in the urine of rats after exposure to chlorobenzene (Krewet *et al.,* 1989 as cited by the Criteria group for occupational standards 2003). Intraperitoneal administration of chlorobenzene to mice induced a dose related increase in the formation of micronucleated polychromatic erythrocytes in the femoral bone marrow (Mohtashamipur *et al*., 1987 as cited by ICPS, 1991). Negative in an oral micronucleus test (Feldt, 1985 as cited by BUA, 1990). Chlorobenzene was also negative in a third micronucleus test in the erythrocytes of the bone marrow of mice after three daily intraperitoneal injections (Shelby *et al.,* 1993). Negative results were also obtained in an oral dominant lethal test and an oral sister chromatid exchange test in the mouse (Feldt, 1985 as cited by BUA, 1990). Chlorobenzene was found negative in two recessive lethal tests in *Drosophila melanogaster* using inhalation exposure (Valencia, 1982 as cited by BUA, 1990). Chlorobenzene was positive in a Comet assay under alkaline conditions on peripheral lymphocytes from mice treated three times on consecutive days with 750 mg/kg bw/day intraperitoneal but negative after single treatment and negative for bone marrow cells (Vaghef and Hellman, 1995).

The *in vivo* studies indicate that under certain specified conditions and at sufficiently high concentrations chlorobenzene has some potential for DNA damage. The results of the chromosome mutation tests were contradictive. A mutagenic potential *in vivo* for chlorobenzene cannot be excluded.

In vivo studies on humans

Major *et al.* (1992) determined the HGPRT mutation frequency in the peripheral blood cells from workers in a factory manufacturing lutidin of chlorobenzene. However, due to exposure to several other aromatic solvents including benzene, these data cannot be used for assessment of the genotoxic effects of chlorobenzene.

Conclusion

The available information indicates that chlorobenzene has some potential to induce DNA damage which is further underpinned by the formation of epoxide-containing metabolites. However, due to the contradictive results on the mutagenic tests *in vitro* and *in vivo*, it is unclear whether the genotoxic activity could represent a risk to human health.

3.5. Carcinogenicity

No inhalation carcinogenicity studies are available for chlorobenzene. There is a gavage study in rats and mice performed by the NTP (1985). The NTP concluded that chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in high dose (120 mg/kg bw/day) male F344/N rats, providing some but not clear evidence of carcinogenicity of chlorobenzene in male rats. Carcinogenic effects of chlorobenzene were not observed in female F344/N rats or in male or female B6C3F1 mice.

3.6. Summary of animal data

Lethality

Only a few animal studies provide adequate LC_{50} values. For male rats, 6-hour LC_{50} value of 2965 ppm was reported by Bonnet *et al*. (1982). For female mice, a 6-hour LC_{50} value of 1886 was reported by Bonnet *et al*. (1978). The dose-response curve in rats was steep: the extrapolated concentration range between 0% to 100% was covered by a factor of approximately 3. However, in mice the dose-response curve was comparatively shallow and was covered by a factor of approximately 10. No deaths were observed in rats and guinea pigs exposed for 30 min to concentrations up to 7970 ppm (UBTL, 1978). Other studies were only limitedly reported or were only available as a summary.

Nonlethal effects

An RD_{50} for sensory irritation of 1054 ppm in mice was assessed for chlorobenzene (Ceaurriz *et al*. 1981). Chlorobenzene was moderately irritating to the skin and not irritating to the eye in standard tests (Mihail, 1984 as cited by BUA, 1990). Slight eye and nasal irritation was observed in rats and guinea pigs exposed to 2990 ppm for 30 min (UBTL, 1978). Eye irritation was also observed after repeated exposure to 3000 ppm in the rat (John *et al.* 1984).

Similar to many other volatile organic compounds, exposure to sufficiently high concentrations of chlorobenzene can induce signs of CNS depression. The CNS effects are dose-related in a continuum of slight effects (lightheadedness) to narcosis and eventually death due to paralysis of the respiratory center.

Animal data related to neurotoxicity are available as clinical effects observed in standard studies and as measurements of specific parameters in specific studies. Some information on the narcotic effects of inhalation exposure of cats to chlorobenzene is available. Ataxia and narcosis was reported in most rats and all guinea pigs exposed to 5850 ppm for 30 min; the effects occurred earlier in guinea pigs than in rats (UBTL, 1978). Several clinical effects indicative of neurotoxicity were reported in the acute toxicity study on rats by Bonnet *et al* (1982). However, no information on the concentrations at which these effects were observed was provided. Frantik *et al*. (1994) studied chlorobenzene exposure in relation to the duration of the tonic extension of the hind limbs after a short electrical pulse in rats and mice. In both species an effect of 30 or 37.5%, respectively was found at 610 ppm (4-hour). This effect is considered a relatively mild endpoint. Rebert *et al.* (1995) determined the concentration needed for auditory changes in rats at 1500 ppm (5 days, 8 hours/day) and the NOEL at 1000 ppm. De Ceaurriz *et al.* (1983) determined the concentration needed for a 50% decrease in duration of immobility in a "behavioral despair" swimming test in mice at 804 ppm (4-hour). This effect is considered a relatively mild endpoint.

Developmental toxicity

The developmental test of chlorobenzene in rats and rabbits (John *et al.* 1984) indicates that chlorobenzene does not induce structural irreversible effects in animals when tested up to maternally toxic concentrations of 590 ppm (6-hour). In rats, an increase in fetal skeletal variations such as delayed ossification was found at the highest exposure concentration of 590 ppm.

A 2-generation test of chlorobenzene in rats (Nair *et al.* 1987) indicates that chlorobenzene does not influence fertility after exposures up to concentrations inducing systemic toxicity in the parents of 450 ppm (6-hour).

Genotoxicity

In vitro studies indicate that chlorobenzene does not induce DNA damage or gene mutations in bacterial tests. Contradicting results were found in gene mutation tests on yeast and DNA damage and gene mutation tests using mammalian cells. The only *in vitro* test on chromosome mutations was negative. Based on the *in vitro* tests, DNA damage and gene mutations cannot be excluded.

In vivo studies indicate that chlorobenzene has some potential for DNA damage. The results of the chromosome mutation tests were contradictive. A mutagenic potential *in vivo* for chlorobenzene cannot be excluded.

Overall, the available information indicates that chlorobenzene has some potential to induce DNA damage perhaps associated with the formation of epoxide-containing metabolites. However, due to the contradictive results on the mutagenic tests *in vitro* and *in vivo*, it is unclear whether the potential measured in some of the studies represents a risk to human health.

Carcinogenicity

The carcinogenicity of chlorobenzene was only tested in an oral study in rats and mice (NTP 1985). NTP concluded that chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in high dose (120 mg/kg bw/day) male F344/N rats, providing some but not clear evidence of carcinogenicity of chlorobenzene in male rats. Carcinogenic effects of chlorobenzene were not observed in female F344/N rats or in male or female B6C3F1 mice.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Absorption

Ogata and Shimada (1983) determined the metabolites excreted in the urine (working hours) of two workers exposed to 0.84 ppm for 415 minutes or 0.5 ppm for 228 minutes and compared it to the estimated intake.

Knecht and Woitowitz (2000) exposed 8 volunteers to 10 ppm for 8 hours/day with an interruption of 45 minutes for 5 successive days to determine a biological tolerance level. The chlorobenzene concentration in the blood was determined at the end of each day and for every 10-minutes during 3.5 hours after the last exposure. In one volunteer the blood level was determined every hour up to four hours during exposure. Further, the concentration of some metabolites in the urine (24-h) was determined. The chlorobenzene level in blood reached the steady state level within one hour during a four hour exposure to 10 ppm. No estimate of pulmonary absorption was provided.

Distribution

Sullivan *et al.* (1983 as cited by ATSDR, 1990) determined the distribution of ^{14}C-labeled chlorobenzene in rats after single or repeated 8-hour inhalation exposures. The radioactivity in all tissues, except fat, increased in proportion to the increase in exposure concentration. The amount of radiolabel in fat increased 30-fold between 100 and 700 ppm. Directly after exposure radioactivity was highest in fat tissues. The preferential distribution of chlorobenzene to the adipose tissue reflects the lipophilic nature of this compound. The preference for distribution towards the adipose tissue was confirmed by Shimada (1988a, in Japanese) in a study in mice after inhalation exposure.

Metabolism

The metabolism of chlorobenzene has been investigated in several species(BUA, 1990; IPCS; 1991; ATSDR, 1990). The first step in the metabolism of chlorobenzene is hepatic oxidation by cytochrome P450 mainly to chlorobenzene-3,4-epoxide but also chlorobenzene-2,3-epoxide and 3-chlorophenol. The epoxides are converted enzymatically by glutathione-transferase to water-soluble mercapturic acid derivates and by epoxide hydratase via dihydrodihydroxychlorobenzene to chlorocathechols. Non-enzymatic rearrangement of the epoxides results in the formation of chlorophenols. The chlorophenols and chlorocathechols can be eliminated in the urine directly or after conjugation with glucuronic acid or sulfate. There was some indication of saturation of the metabolism of inhaled chlorobenzene in rats at 400 ppm. The metabolism can be affected by pre-treatment with microsomal enzyme-inducing agents and by glutathione depletion. Binding of chlorobenzene to protein, RNA and DNA was shown in several studies and were probably caused by the reactivity of the epoxide (BUA, 1990).

Excretion

Chlorobenzene is eliminated unchanged via the lungs and as metabolites principally in the urine and to a smaller extent in the feces. The main metabolites in the urine are 4-chlorocatechol conjugates and 4-chlorophenylmercapturic acid. The percentage eliminated via the lungs increases with dose. The

elimination of inhaled chlorobenzene consisted of a quick first phase (probably exhalation of chlorobenzene) and a second slower phase (probably metabolism and urinary excretion) (ATSDR 1990).

PBPK-modeling

Recently, a physiologically based pharmacokinetic model was developed by Thrall *et al.* (2004) for the respiratory absorption, distribution, metabolism and elimination of chlorobenzene in the rat and evaluated against real-time exhaled breath data.

A physiologically based pharmacokinetic model was developed by Kumagai and Matsunaga (1995) to relate the inhalation exposure of workers to the urinary excretion of 4-chlorocatechol. The model was compared to data reported by others. The results indicate that the pharmacokinetic model can be used to estimate the urinary concentrations of 4-chlorocatechol.

4.2. Mechanism of Toxicity

Acute inhalation exposure to chlorobenzene results in contact irritation and CNS depression. Repeated inhalation exposure results also in toxicity to several organs including liver, kidney and white blood cells.

As with many volatile organic compounds (VOCs), the concentration of chlorobenzene in the brain is probably the pivotal factor for CNS depression. According to De Jongh *et al*. (1998), primarily the calculated concentration in the lipid phase of the brain shows a good correlation with acute mortality data for several VOCs. Taking into account kinetics, it was shown that LC_{50} values for 15 compounds correlated to a concentration of 70 ± 31 mM of these compounds in brain lipids (De Jongh *et al*.1998). So, most likely the concentration of chlorobenzene in the brain lipid phase determines the potential for CNS depression and – eventually – death by paralysis of the respiratory center.

No information is available on the mechanism of the toxicity to several organs including liver, kidney and white blood cells after inhalation exposure. However, studies (Reid 1973a, b) with intraperitoneal exposure to radioactive chlorobenzene show covalent binding to proteins. Also, pre-treatment with cytochrome P450 inhibitors or inducers reduced or potentiated the binding and the renal and hepatic necrosis. This suggests that the toxicity to liver and kidney and probably also the toxicity to the white blood cells (or bone marrow) is due to reactive metabolites. The reactive epoxides are normally removed by the enzymatic reaction with glutathione or by epoxide hydratase. Repeated exposure can therefore result in glutathione depletion. A decrease in glutathione can result in an increase in covalent binding and toxicity. This would indicate that repeated exposure can have more severe effects than a single exposure.

The mechanism of action for the toxicity of chlorobenzene is unknown for several effects and therefore the appropriate dose metric cannot be assessed. PBPK-modeling could provide some additional insight by evaluating correlations between selected dose metrics and dose-response data from toxicity studies. However, as of the date of the present analysis not all metabolites can be modeled in sufficient detail and the available data are scarce for appropriate dose-response modeling. Therefore, at present the uncertainty in using the available PBPK-modeling is too large to be adequate for use within the present AEGL-framework.

4.3. Structure Activity Relationships

No quantitative structure activity relationships were found for chlorobenzeneand its congeners. However, chlorobenzene shares its CNS depressing action with other aromatic compounds like benzene, toluene and xylene. When comparing the LC_{50} values for several aromatic compounds as were tested by Bonnet *et al.* (1979, 1982), chlorobenzene was one of most toxic compounds. Only dichlorobenzene was more toxic.

4.4. Other relevant information

4.4.1. Irritation and Sensitization

Chlorobenzene was considered moderately irritating to the skin and non-irritating to the eye in the OECD 404 and 405 tests (Suberg 1983 as cited by BUA 1990). A maximization test gave no indication of a sensitizing potential (Mihail 1984 as cited by BUA 1990).

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of human data relevant to AEGL-1

Ogata *et al*. (1991) exposed four volunteers to a chlorobenzene concentration of 60 ppm for 3 hours in the morning and 4 hours in the afternoon with a 1-hour break in between. All volunteers complained of a sensation of disagreeable odor and of drowsiness after cessation of exposure. Three of four had a heavy feeling in the head and/or headache, two out of four a throbbing pain in the eyes and one out of four complained of a sore throat. No information was given about the time of onset of these complaints. Exposure did not affect pulse rates and systolic and diastolic pressure. Flicker fusion frequency values were reduced significantly at the end of exposure period of 3 hours in the morning, indicating lowered perception. No further effect was seen in the afternoon. The significance of this finding is difficult to interpret.

Knecht and Woitowitz (2000) exposed eight volunteers in a chamber to 10 ppm for 8 hours a day for 5 days to determine the relation between exposure and urinary 4-chlorocatechol and chlorophenols. None of thesubjects made a complaint. This especially applied to chemical irritation of eyes and the nasal region, respiratory tract or skin, allergic or dermal reactions or acute central nervous system depressant with signs of headache, dizziness or fatigue (Knecht personal communication, 2005).

5.2. Summary of animal data relevant to AEGL-1

No adequate relevant animal data that address the level of effects defined by the AEGL-1 were retrieved.

5.3. Derivation of AEGL-1

The effects described in the study by Ogata *et al.* (1991) at 60 ppm are indicative of slight CNS depression and of local irritation and considered to represent discomfort. These effects were not observed during or after exposure of humans to 10 ppm (8-hour) in the study of Knecht and Woitowitz (2000). It is proposed to use 10 ppm (8-hour) as a conservative point of departure for the derivation of AEGL-1 values. Because human data are used, the interspecies uncertainty factor is 1. An uncertainty factor for intraspecies variation could be considered because the studies were performed with only 4 (Ogata *et al.*, 1991) or 8 volunteers (Knecht and Woitowitz, 2000). However, the effects seen at 60 ppm are considered to be rather slight and it cannot be ruled out that odor might have interfered. Therefore, some safety margin is already present when using 10 ppm as point of departure. Furthermore, Knecht and Woitowitz exposed their volunteers for 5 consecutive days. Therefore, the (8-hour) 10 ppm exposure level is considered to be a conservative point of departure, hence an UF of 1 is used for intraspecies variation. This results in an 8-hour AEGL-1 value of 10 ppm. No information on the time-dependency of the effects at 10 or 60 ppm is available. Since the described effects at 60 ppm include irritation and CNS depression, the 8-hour AEGL-1 value of 10 ppm is considered appropriate for all time-points. It is further noted that Knecht and Woitowitz (2000) reported that the chlorobenzene levels in blood reached a steady-state level within one hour. This supports the same AEGL-1 value for all time points.

TABLE 4. AEGL-1 Values for Chlorobenzene				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of human data relevant to AEGL-2

No human data are available that adequately address toxicity endpoints as defined by AEGL-2. Ogata *et al*. (1991) exposed four volunteers to a chlorobenzene concentration of 60 ppm for 3 hours in the morning and 4 hours in the afternoon with a 1-hour break in between. All volunteers complained of a sensation of disagreeable odor and of drowsiness after exposure. Three out of four had a heavy feeling in the head and/or headache, two out of four a throbbing pain in the eyes and one out of four a sore throat. Exposure did not affect pulse rates and systolic and diastolic pressure. Flicker fusion values were significantly decreased at the end of exposure period of 3 hours in the morning, indicating lowered perception. No further effect was seen in the afternoon.

Knecht and Woitowitz (2000) exposed eight volunteers to 10 ppm for 8 hours a day for 5 days to determine the relation between exposure and urine levels of 4-chlorocatechol and chlorophenols. None of the probands made complaints. This especially applied to chemical-irritative effects as irritation of eyes and the nasal region, respiratory tract or skin, allergic or dermal reactions or acute central nervous system depressant with signs of headache, dizziness or fatigue (Knecht personal communication, 2005).

6.2. Summary of animal data relevant to AEGL-2

Whole body exposure of groups of 5 guinea pigs per sex and of 5 rats per sex induced slight eye and nasal irritation at a mean analytical concentration of 2990 (SD: 53) but none of the animals was judged to suffer from impaired ability to escape. At the next higher concentration, 5850 ppm(1350) all guinea pigs and most rats suffered from narcosis and were judged to have impaired ability to escape (UBTL, 1978). Frantik *et al.* (1994) determined that a 37.5% decrease in the duration of tonic extension of the hind limbs after a short electrical pulse was induced after a 4-hour exposure of rats to 611 ppm. A comparable study in mice determined a 30% effect level of 610 ppm. These concentrations were not expected to influence normal locomotor activity or to induce behavioral excitation. Therefore, these concentrations are considered as sub AEGL-2 concentrations. Rebert *et al.* (1995) determined a NOEL in the rat for the auditory system of 1000 ppm for a 5 day exposure for 8 hours a day. As organic solvents can induce permanent hearing loss, this is considered a relevant endpoint for AEGL-2 derivation. However, whether permanent hearing loss can already be induced by single exposure is unclear. De Ceaurriz *et al.* (1983) determined the ID50 for the decrease in the duration of immobility in the 3-minute "behavioral despair" swimming test at 804 ppm. This effect is considered to be a subtle change in neurobehavior and therefore the ID_{50} to be a sub AEGL-2 concentration.

Repeated exposure of rats and rabbits resulted in severe effects at 1000 ppm and above, in limited effects around 500 ppm and no effects at 200 ppm.

6.3. Derivation of AEGL-2

There are no adequate human data for derivation of AEGL-2. In the study by Ogata *et al.* (1991), it was found that exposure to 60 ppm for 4 or 8 hours induced discomfort. The subtle CNS effects observed in the available single exposure studies with experimental animals are difficult to interpret because it is unclear whether these effects would lead to a decrease in the ability to escape or serious or irreversible health effects. The effects reported by Frantik *et al.* (1994) and De Ceaurriz *et al.* (1993) are considered to represent sub AEGL-2 effects. The most appropriate point of departure is the absence of AEGL-2 related effects in rats and guinea pigs exposed to 2990 ppm for 30 min.

An interspecies UF of 3 is considered sufficient because 1) data were comparable for rats and guinea pigs suggesting no large interspecies differences and 2) the critical effect is CNS depression. The chlorobenzene concentration in the brain is probably related directly to the inhalation rate. Therefore, it is expected that humans require higher external concentrations compared to rodents, to obtain a similar level of chlorobenzene in the blood or brain as is observed for other VOCs (trichloroethylene, toluene). This view is supported in the technical support document for toluene, where kinetic information is available for various species, including humans. Further, the use of a larger interspecies UF (next to an intraspecies UF of 3) would result in AEGL-2 levels that would conflict with human data (i.e. below the 60 ppm which was shown to cause only minor effects in humans (Ogata *et al*., 1991)). Experience with anesthetic gases shows that the interindividual variability for CNS depression caused by these gases will generally not be greater than a factor of 2 to 3. Therefore, an intraspecies UF of 3 is proposed resulting in an overall UF of 10 and a 30-min AEGL-2 of 300 ppm.

The value of 300 ppm for 30 min was extrapolated across time periods using $C^n \times t = k$ with default values $n = 1$ for extrapolation to 1 hour and $n=3$ for extrapolation to 10 min. The relationship between concentration and duration of exposure as related to lethality was examined by Ten Berge et al. (1986) for approximately 20 irritant or systemically-acting vapors and gases. The authors subjected the individual animal data sets to probit analysis with exposure duration and exposure concentration as independent variables. An exponential function ($C^n \times t = k$), where the value of n ranged from 0.8 to 3.5 for different chemicals was found to be an accurate quantitative descriptor for the chemicals evaluated. Approximately 90 percent of the values of n range between n=1 and n=3. Consequently, these values were selected as the reasonable lower and upper bounds of n to use when data are not available to derive a value of n. The 4- and 8-hour AEGL-2 values were set equal to the 1-hour value because 1) chloro-benzene concentrations in blood reach a steady-state within 1 hour and elimination is rapid and 2) time scaling would result in 4- and 8-hour AEGL-2 values (37 and 19 ppm, respectively) that conflict with human data (Ogata *et al*. 1991). The AEGL-2 values are summarized in Table 5.

TABLE 5. AEGL-2 Values for Chlorobenzene

10-minute	30-minute	1-hour	4-hour	8-hour
430 ppm (2021 mg/m^3)	300 ppm (1410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of human data relevant to AEGL-3

No acute human mortality data are available. In the study by Ogata *et al.* (1991), it was found that exposure to 60 ppm for 4 or 8 hours induced discomfort.

7.2. Summary of animal data relevant to AEGL-3

Several relevant studies with experimental animals are available but most of them are only available from secondary sources or are old and only limitedly described. The only study in cats by Gotzmann as cited by Flury and Zernik (1931) is old and the number of animals and the observation period is unknown. Therefore, these results cannot be used. The only acute study in rabbits by Rozenbaum is available as a limited summary from a secondary source. Further, mortality was seen in this study at 550 to 660 ppm although no mortality was found at an even higher dose level in the rabbit of 1000 ppm in the developmental toxicity study by John *et al.* (1984). Therefore, these results cannot be used.

Groups of 5 guinea pigs per sex and 5 rats per sex were whole body exposed to mean analytical concentrations of 2990 (SD: 53), 5850 (1350) or 7970 (355) ppm chlorobenzene for 30 min and held under observation for 14 days. No deaths were observed at any concentration (UBTL, 1978).

Bonnet *et al.* (1982) reports a 6-hour LC_{50} of 2965 ppm with a 95% CI of 2787 - 3169 ppm and a regression line of *probit = -33 + 10.9 logC* for rats. These results are in line with the findings that mortality was observed at 3000 ppm but not at 1000 ppm in a range-finding developmental study in rats with exposure during 7 hours per day. However, the results of Bonnet *et al.* (1982) are not in line with the results of Rebert *et al.* (1995) who did not report deaths in rats exposed for 5 days for 8 hour per day to chlorobenzene concentrations ranging from 1000 to 2400 ppm.

There are several studies in mice but for most studies only a limited summary is available. Therefore, these studies cannot be used. A 6-hour LC_{50} of 1886 ppm was determined with a 95% CI of 1781 - 1980 ppm and a regression line of *probit = -17.06 + 6.734 logC* (Bonnet *et al.*, 1979).

7.3. Derivation of AEGL-3

There are no adequate human data for derivation of AEGL-3. The available human data show that exposure to 60 ppm for up to 8 hours only induces discomfort.

No deaths were reported in rats and guinea pigs (5 animals per sex per species) exposed to chlorobenzene concentrations of up to 7970 ppm for 30 min (UBTL, 1978). Bonnet *et al.*(1979, 1982) reported acute mortality studies with mice and rats. The mouse appeared to be more sensitive to the effects of chlorobenzene as concluded from the lower LC_{50} value and the less steep slope. An LC_{01} of 851 ppm can be calculated from the mouse study using the respective probit equation (probit value of 2.67); the corresponding value for rats would be 1873 ppm. However, the mouse often appears to be a too sensitive species in acute mortality studies.

The data in rats and guinea pigs reported by UBTL (1978) are considered to provide the most appropriate point of departure for AEGL-3 derivation, i.e. no mortality in rats and guinea pigs after exposure to 7970 ppm for 30 min. The rationale for the choice of an interspecies and intraspecies UF (of 3 each) and for time scaling (using default values for n for extrapolation to 10- and 60-min and flatlining to 4- and 8-hours) is similar to that for AEGL-2. Time scaling with n=1 would result in an 8-hour AEGL-3 value of 50 ppm while only slight CNS depression and irritation occurred in humans exposed to 60 ppm for 7 hours. Table 6 summarizes the AEGL-3 values. These values are considered to be consistent with the AEGL-2 values (Table 5) and supported by the 6-hour LC_{01} of 1873 ppm calculated from the probit equation reported by Bonnet *et al*. (1982).

TABLE 6. AEGL-3 Values for Chlorobenzene				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
1100 ppm (5170 mg/m^3)	800 ppm (3760 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)

8. SUMMARY OF AEGLS

8.1. AEGL values and toxicity endpoints

TABLE 7. Summary of AEGL Values					
Classification	**Exposure Duration**				
	10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
AEGL-1 (Nondisabling)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)
AEGL-2 (Disabling)	430 ppm (2021 mg/m^3)	300 ppm (1410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)
AEGL-3 (Lethal)	1100 ppm (5170 mg/m^3)	800 ppm (3760 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)

8.2. Comparison with other standards and guidelines

The IDLH of 1000 ppm was based on acute inhalation toxicity studies also discussed in this document and, with exception of the 10-min AEGL-3 value, all AEGL-values are below the IDLH. The 8-hour AEGL-2 value is about twice as high as the PEL-TWA while the TLV-TWA, MAK and MAC values are equal to the AEGL-1 values.

TABLE 8. Extant Standards and Guidelines for Chlorobenzene					
Guideline	**Exposure Duration**				
	10 minute	**30 minute**	**1 hour**	**4 hour**	**8 hour**
AEGL-1	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m^3)
AEGL-2	430 ppm (2021 mg/m^3)	300 ppm (1410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)
AEGL-3	1100 ppm (5170 mg/m^3)	800 ppm (3760 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)
ERPG-1 (AIHA)[a]			30 ppm		

ERPG-2 (AIHA)			500 ppm		
ERPG-3 (AIHA)			1000 ppm		
EEGL (NRC)[b]					
PEL-TWA (OSHA)[c]					75 ppm (350 mg/m^3)
PEL-STEL (OSHA)[d]					
IDLH (NIOSH)[e]		1000 ppm			
REL-TWA (NIOSH)[f]					
REL-STEL (NIOSH)[g]					
TLV-TWA (ACGIH)[h]					10 ppm
TLV-STEL (ACGIH)[i]					
MAK (Germany)[j]					10 ppm (46 mg/m^3)
MAK Peak Limit (Germany)[k]					
MAC (The Netherlands)[l]					10 ppm (46 mg/m3, MAC-TGG)

[a]**ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association** (AIHA 1994)
The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor.
The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual=s ability to take protection action.
The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects.

[b]**EEGL (Emergency Exposure Guidance Levels, National Research Council** (NRC 1985)
The EEGL is the concentration of contaminants that can cause discomfort or other evidence of irritation or intoxication in or around the workplace, but avoids death, other severe acute effects and long-term or chronic injury.

[c]**OSHA PEL-TWA (Occupational Safety and Health Administration, Permissible Exposure Limits - Time Weighted Average)** (OSHA 19??) is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[d]**OSHA PEL-STEL (Permissible Exposure Limits - Short Term Exposure Limit)** (OSHA 199?) is defined analogous to the ACGIH-TLV-STEL.

[e]**IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH 199?) represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects.

[f]**NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average)** (NIOSH 199?) is defined analogous to the ACGIH-TLV-TWA.

[g]**NIOSH REL-STEL (Recommended Exposure Limits - Short Term Exposure Limit)** (NIOSH 199?) is defined analogous to the ACGIH TLV-STEL.

[h]**ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** (ACGIH 199?) is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[i]**ACGIH TLV-STEL (Threshold Limit Value - Short Term Exposure Limit)** (ACGIH 199?) is defined as a 15-minute TWA exposure which should not be exceeded at any time during the workday even if the 8-hour TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 minutes and should not occur more than 4 times per day. There should be at least 60 minutes between successive exposures in this range.

[j]**MAK (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration])** (Deutsche Forschungsgemeinschaft [German Research Association] 2000) is defined analogous to the ACGIH-TLV-TWA.

[k]**MAK Spitzenbegrenzung (Peak Limit [give category])** (German Research Association 2000) constitutes the maximum average concentration to which workers can be exposed for a period up to 30 minutes with no more than 2 exposure periods per work shift; total exposure may not exceed 8-hour MAK.

[l]**MAC (Maximaal Aanvaaarde Concentratie [Maximal Accepted Concentration])** (SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment], The Hague, The Netherlands 2000) is defined analogous to the ACGIH-TLV-TWA.

8.3. Data quality and research needs

Human data are very scarce, only two human kinetic studies were retrieved but no adequate on effects in human were available. Studies aimed at studying toxic effects addressed by AEGL-2 are limited to one study of sufficient quality in which rats and guinea pigs were exposed for 30 min to nonlethal concentrations.

9. REFERENCES

Anonymous. 1994. Toxicity and health hazard summary of chlorobenzene with cover letter dated 04/05/94. EPA/OTS; Doc #86940000289. NTIS/OTS0572392.

Aranyi, C., O'Shea W. J., Graham J. A. and F.J. Miller. 1986 The Effects of Inhalation of Organic Chemical Air Contaminants on Murine Lung Host Defenses. Fund. Appl. Toxicol. 6: 713-20.

ATSDR (Agency for Toxic Substances and Diseases Registry). 1990. Toxicological Profile for Chlorobenzene." U.S. Department of Health and Human Services. TP-90-06. December, 1990.

Ten Berge, W.F., Zwart, A. and LM Appelman. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. J. Haz. Mat. 13(3):301-309.

Bonnet, P., Raoult G. and D. Gradiski. 1979. Concentrations Léthales 50 Des Principaux Hyrocarbures

Aromatiques. Arch. Mal. Prof.. 40:805-10.

Bonnet, P. Y., Morele G., Raoult G., Zissu D. and D. Gradiski. 1982. Determination of the Median Lethal Concentration of the Main Aromatic Hydrocarbons in the Rat. Arch. Mal. Prof. 43(3):461-65.

BUA (Advisory Board for Environmental Relevant Waste of the German Chemical Society). 1990. "Chlorobenzene. BUA Substance Report 54 (November 1990)." (VCH: Weinheim, Fed. Rep. Ger.)

Criteria group for occupational standards. 2003. Scientific Basis for Swedish Occupational Standards. XXIV. Consensus Report for Chlorobenzene. Arbete Och Halsa. 16:48-54.

De Ceaurriz, J. C., Micillino J. C., Bonnet P. and J. P. Guenier. 1981. Sensory Irritation Caused by Various Industrial Airborne Chemicals. Toxicol. Lett. 9:137-43.

De Ceaurriz, J., Desiles J. P., Bonnet P., Marignac B., Muller J. and J.P. Buenier. 1983. Concentration-Dependent Behavioral Changes in Mice Following Short-Term Inhalation Exposure to Various Industrial Solvents. Toxicol. Appl. Pharmacol. 67:383-89.

De Jongh, J., Verhaar, H.J.M. and J.L.M. Hermens. 1998. Role of kinetics in acute lethality of nonreactive volatile organic compounds (VOCs). Tox. Sci. 45:26-32.

Dilley, J. V. 1977. Toxic Evaluation of Inhaled Chlorobenzene (Monochlorobenzene). United States Environmental Protection Agency , no. PB-276 623: 71-80.

Flury, F. and F. Zernik. 1931 Schadliche Gase. Springer, Berlin.

Frantik, E., Hornychova, M., and M. Horvath. 1994. Relative Acute Neurotoxicity of Solvents: Isoeffective Air Concentrations of 48 Compounds Evaluated in Rats and Mice. Environ. Res. 66(2):173-85.

Hayes, W. C,. Gushaw T. S., Hohnson K. A., Hanley T. R., Ouellette J. H. and J.A. John. 1981. Monochlorobenzene Inhalation Teratology Study in Rats and Rabbits. Unpublished report. Toxicology Research Laboratory, Dow Chemical Company.

Hellman, B. 1993. NIOH and NIOSH Basis for an Occupational Health Standard: Chlorobenzene. Publications Dissemination, DSDTT, National Institute for Occupational Safety and Health (NIOSH), Jan. 1993.

IPCS (International programme on chemical safety) 1991. Chlorobenzenes other than hexachlorobenzene. WHO; Environmental Health Criteria 128. WHO Geneva.

IRPTC (International Register of Potentially Toxic Chemicals). 1988. Chlorobenzenes (chlorobenzene, dichlorobenzene trichlorobenzene). Scientific Reviews of Soviet Literature of Toxicity and Hazards of Chemicals No 108. Moscow

John, J. A., Hayes W. C., Hanley T. R. jr., Johnson K. A., Gushow T. S. and K.S. Rao. 1984. Inhalation Teratology Study on Monochlorobenzene in Rats and Rabbits. Toxicol. Appl. Pharmacol. 76:365-73.

Khalil, A. M., and M. M. T. Odeh. 1994. Genetic Toxicology of Benzene and Its Derivatives in Rat Bone Marrow Cell Cultures. Toxicol. Environ. Chem.;45(3+4):157-66.

Knecht, U., and H. J. Woitowitz. 2000. Human Toxicokinetics of Inhaled Monochlorobenzene: Latest Experimental Findings Regarding Re-Evaluation of the Biological Tolerance Value. Int. Arch. Occup.

Environ. Health 73(8):543-54.

Kumagai, S., and I. Matsunaga. 1995. Effect of Variation of Exposure to Airborne Chlorobenzene on Internal Exposure and Concentration of Urinary Metabolite. Occup. Environ. Med. 52(1):65-70.

Major, J., Kemeny, G. and A. Tompa. 1992. Genotoxic Effects of Occupational Exposure in the Peripheral Blood Lymphocytes of Pesticide Preparing Workers in Hungary. Acta Med Hung. 49(1-2): 79-90.

Nair, R. S., Barter, J.A., Schroeder, R.E., Knezevich A. and C. R. STACKl. 1987. 2-Generation Reproduction Study With Monochlorobenzene Vapor in Rats. Fund. Appl. Toxicol. 9:678-86.

NIOSH (National Institute for Occupational Safety and Health). 2005. NIOSH pocket guide to chemical hazards. HTTP:/www/cdc.gov/niosh/npg/npgd0121.html.

NTP. 1985. Toxicology and Carcinogenesis Studie of Chlorobenzene in F344/N Rats and B6C3F1 Mice (Gavage Studies)." US Department of Health and Human Services, Public Health Service, National Institutes of Health NTP No. 261 NIH Publ.No. 83-2517.

Ogata, M., Taguchi, T., Hirota, N., Shimada, Y. and S. Nakae. 1991. Quantitation of Urinary Chlorobenzene Metabolites by HPLC: Concentrations of 4-Chlorocatechol and Chlorophenols in Urine and of Chlorobenzene in Biological Specimens of Subjects Exposed to Chlorobenzene. Int Arch. Occup. Environ. Health 63(2):121-8.

Ogata, M. and Y. Shimada. 1983 Differences in urinary monochlorobenzene metabolites between rats and humans. Int. Arch. Occup. Environ. Health 53:51-57.

Rebert, C. S., Schwartz, R. W., Svendsgaard, D. J., Pryor, G. T. and W. K. Boyes. 1995 Combined Effects of Paired Solvents on the Rat's Auditory System. Toxicology 105(2-3):345-54.

Reid, W.D. and G. Krishna. 1973a. Centrolobular hepatic necrosis related to covalent binding of metabolites of halogenated aromatic hydrocarbons. Exp. Mol. Path. 18:80-99.

Reid, W.D. 1973b. Mechanism of renal necrosis induced by bromobenzene or chlorobenzene. Exp. Mol. Path. 19:197-214.

Ruth, J. H. 1986. Odor tresholds and irritation levels: a review. Am. Ind. Hyg. Assoc. J. 47:142-151.

Shelby, M D, Erexson, G. L., Hook, G. J. and R R Tice. 1993. Evaluation of a Three-Exposure Mouse Bone Marrow Micronucleus Protocol: Results With 49 Chemicals. Environ. Mol. Mutagen. 21(2): 160-79.

Shimada, Y. 1988a. Studies on Monochlorobenzene Poisoning: Part III. Distribution of Monochlorobenzene in the Organs of Pregnant Mice and Transfer to the Fetus Through the Placenta: Comparison With Trichloroethylene and 1, 1, 1-Trichloroethane. Okayama Igakkai Zasshi 100(1-2):147-154.

Shimada, Y. 1988b. Studies on monochlorobenzene poisoning: Part II. Distribution of monochlorobenzene among the organs of mice. Okayama Igakkai Zasshi 100(1-2): 135-146.

Ten Berge, W.F., Zwart, A. and LM Appelman. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. J. Haz. Mat.. 13(3):301-309.

Thrall, K. D., Woodstock, A. D. and M. R. Kania. 2004. Development of a Physiologically Based Pharmacokinetic Model for Chlorobenzene in F-344 Rats. J. Toxicol. Environ. Health A 67(7):525-36.

UBTL 1978. Utah Biomedical Test Laboratory Report on NIOSH sponsored inhalation study for IDLH values (Final report). With cover letter dated 102291. U.S. EPA/OPTS Public Files OTS0534605.

US EPA, 1995. http://www.epa.gov/chemfact/chlor-sd.pdf

Vaghef, H. and B. Hellman. 1995. Demonstration of chlorobenzene-induced DNA damage in mouse lymphocytes using the single cell gel electrophoresis assay. Toxicology 96(1):19-28.

APPENDIX A: Derivation of AEGL Values

Derivation of AEGL-1

Key study:	Ogata et al., 1991 and Knecht and Woitowitz, 2000
Toxicity Endpoint:	Slight CNS depression and local irritation at 60 ppm (3 plus 4 hours) and none at 10 ppm (8 hours/day for 5 days)
Time scaling:	None, because of the nature of the effects
Uncertainty factors:	1
Calculations:	None
10-minute AEGL-1	10 ppm (47 mg/m^3)
30-minute AEGL-1	10 ppm (47 mg/m^3)
1-hour AEGL-1	10 ppm (47 mg/m^3)
4-hour AEGL-1	10 ppm (47 mg/m^3)
8-hour AEGL-1	10 ppm (47 mg/m^3)

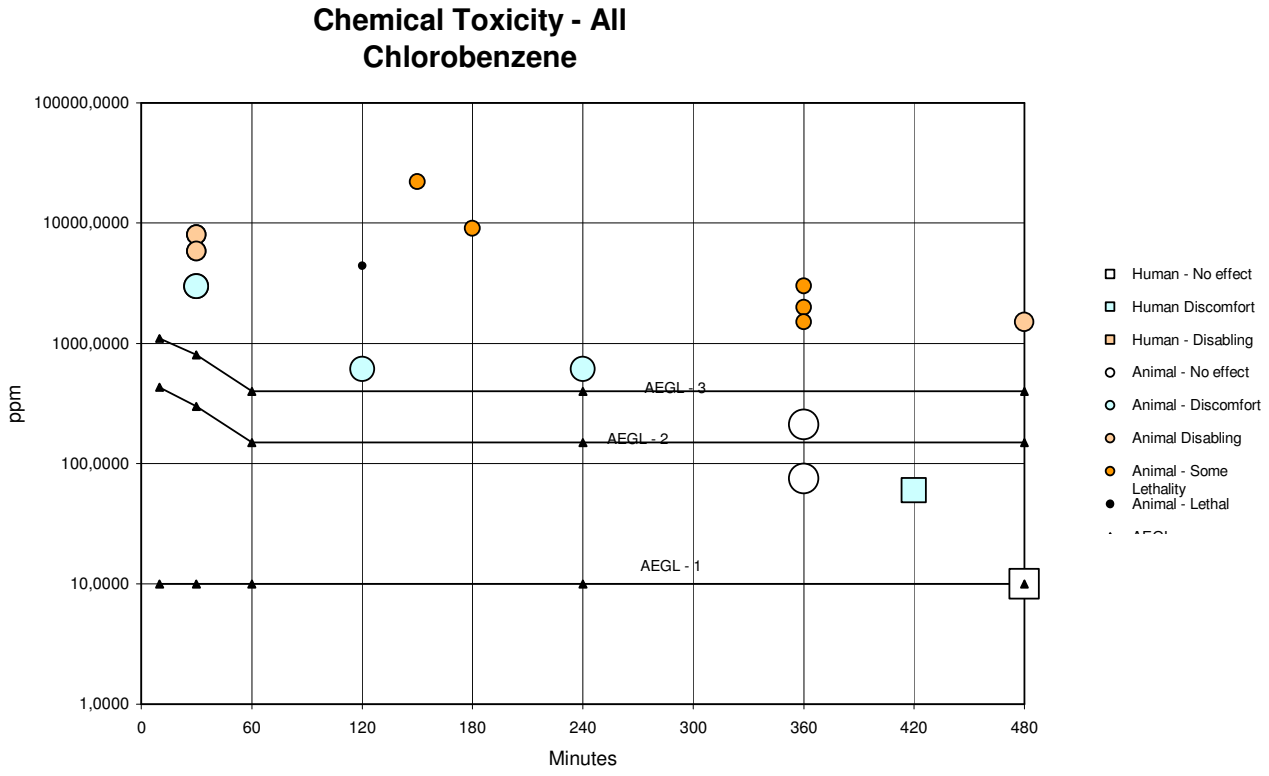
Derivation of AEGL-2

Key study:	UBTL, 1978
Toxicity Endpoint:	Narcosis in rats and guinea pigs
Time scaling:	The value of 2990 ppm for 30 min was extrapolated across time periods using $C^n \times t = k$ with default value n = 1 for extrapolation to 10 min and n=3 for extrapolation to 1 hour. The value for 4- and 8-hours were set equal to the 1-hour value because a steady-state chlorobenzene concentration in blood is reached within 1 hour and for reasons of consistency with human data.
Uncertainty factors:	interspecies 3, intraspecies 3
Calculations:	To 10 min: $C^3 * t = k$ $k = (2990 \text{ ppm})^3 * 30 \text{ min} = 8.02 * 10^{11} \text{ ppm}^3 \text{ min}$ To 1 hour: $C * t = k$ $k = 2990 \text{ ppm} * 30 \text{ min} = 89{,}700 \text{ ppm min}$
10-minute AEGL-2	$(8.02 * 10^{11} \text{ ppm}^3 \text{ min} / 10 \text{ min})^{1/3} / 10 = 431 \text{ ppm}$
30-minute AEGL-2	2990 ppm / 10 = 299 ppm
1-hour AEGL-2	(89,700 ppm min / 60) / 10 = 150 ppm
4-hour AEGL-2	equal to 1-hour AEGL-2: 150 ppm
8-hour AEGL-2	equal to 1-hour AEGL-2: 150 ppm

Derivation of AEGL-3

Key study:	UBTL, 1978
Toxicity Endpoint:	No mortality in rats and guinea pigs
Time scaling:	The value of 7970 ppm for 30 min was extrapolated across time periods using $C^n \times t = k$ with default value n = 1 for extrapolation to 10 min and n=3 for extrapolation to 1 hour. The value for 4- and 8-hours were set equal to the 1-hour value because a steady-state chlorobenzene concentration in blood is reached in 1 hour and for reasons of consistency with AEGL-2 values.
Uncertainty factors:	interspecies 3, intraspecies 3
Calculations:	To 10 min: $C^3 * t = k$ $k = (7970 \text{ ppm})^3 * 30 \text{ min} = 1.52 * 10^{13} \text{ ppm}^3 \text{ min}$ To 1 hour: $C * t = k$ $k = 7970 \text{ ppm} * 30 \text{ min} = 239{,}100 \text{ ppm min}$
10-minute AEGL-3	$(1.52 * 10^{13} \text{ ppm}^3 * \text{min} / 10 \text{ min})^{1/3} / 10 = 1149 \text{ ppm}$
30-minute AEGL-3	7970 ppm / 10 = 797 ppm
1-hour AEGL-3	(239,100 ppm * min / 60 min) / 10 = 399 ppm
4-hour AEGL-3	equal to 1-hour AEGL-3: 399 ppm
8-hour AEGL-3	equal to 1-hour AEGL-3: 399 ppm

APPENDIX B: Category plot for chlorobenzene


Chemical Toxicity - All
Chlorobenzene
100000,0000
10000,0000
1000,0000
100,0000
10,0000
1,0000
ppm
0
60
120
180
240
300
360
420
480
Minutes
AEGL - 3
AEGL - 2
AEGL - 1
Human - No effect
Human Discomfort
Human - Disabling
Animal - No effect
Animal - Discomfort
Animal Disabling
Animal - Some Lethality
Animal - Lethal

1

APPENDIX C: Derivation Summary for Chlorobenzene AEGLs

ACUTE EXPOSURE GUIDELINE LEVELS FOR CHLOROBENZENE (CAS Reg. No. 108-90-7) DERIVATION SUMMARY

AEGL-1 VALUES

10-minute	30-minute	1-hour	4-hour	8-hour
10 ppm (47 mg/m3)	10 ppm (47 mg/m3)	10 ppm (47 mg/m^3)	10 ppm (47 mg/m3)	10 ppm (47 mg/m^3)

Key Reference: Ogata et al., 1991 (1); Knecht and Woitowitz, 2000 (2)

Test Species/Strain/Number: Humans (1): n=4 (2): n=8

Exposure Route/Concentrations/Durations: inhalation (1): 60 ppm, 7-h exposure with a 1-h break after 3 hours (2): 10 ppm, 8 h/day for 5 days

Effects:
10 ppm None (2)
60 ppm Slight CNS depression and local irritation (1)

Endpoint/Concentration/Rationale: Slight CNS depression and local irritation (1) at 60 ppm (7-h exposure with a 1-h break after 3 hours) was considered to represent discomfort.

Uncertainty Factors/Rationale:
Total uncertainty factor: 1
Interspecies: factor of 1 because the effects were seen in a human volunteer study
Intraspecies: factor of 1 because the effects at 60 ppm were only very slight

Modifying Factor: None

Animal to Human Dosimetric Adjustment: Not relevant

Time Scaling: None. No information on time dependency is available in the studies. The observed effects do not indicate a strong time dependency which is confirmed by the absorption data.

Data Adequacy: Studies were kinetic studies and were not specifically designed to determine the presence or absence of toxicity.

AEGL-2 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
430 ppm (2021 mg/m^3)	300 ppm (1410 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)	150 ppm (705 mg/m^3)

Key Reference: UBTL, 1978

Test Species/Strain/Number: rats and guinea pigs (5 per sex per species)

Exposure Route/Concentrations/Durations: 30-min exposures to 2990, 5850, or 7970 ppm; 14-day observation

Effects:
2990 ppm: slight eye and nasal irritation;
5850 ppm: narcosis; impaired ability to escape
7970 ppm: no deaths, ataxia and narcosis

Endpoint/Concentration/Rationale: No narcosis at 30-min exposure to 2990 ppm, no animals suffered from impaired ability to escape.

Uncertainty Factors/Rationale:
Total uncertainty factor: 10; AEGL-2 values derived with a larger UF would conflict with human data.
Interspecies: factor of 3 because 1) data were comparable for rats and guinea pigs suggesting no large interspecies differences and 2) the critical effect is CNS depression.
Intraspecies: factor of 3 because interindividual variability for CNS depression by comparable gasses generally will not be greater than a factor of 2 to 3.

Modifying Factor: None

Animal to Human Dosimetric Adjustment: None

Time Scaling: Default values of 3 and 1 to 10-min and 60-min, respectively. AEGL-2 values for 4- and 8-hours are set equal to the 1-hour value because 1) chlorobenzene concentrations in blood reach a steady-state within 1 hour and elimination is rapid and 2) time scaling would result in 4- and 8-hour AEGL-2 values that would conflict with human data.

Data Adequacy: Data are limited to one study (30 min to 3 concentrations) aimed to derive an IDLH value.

1

AEGL-3 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
1100 ppm (5170 mg/m^3)	800 ppm (3760 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)	400 ppm (1880 mg/m^3)

Key Reference: UBTL, 1978

Test Species/Strain/Number: rats and guinea pigs (5 per sex per species)

Exposure Route/Concentrations/Durations: 30-min exposures to 2990, 5850, or 7970 ppm; 14-day observation

Effects:
2990 ppm: slight eye and nasal irritation;
5850 ppm: narcosis; impaired ability to escape
7970 ppm: no deaths, ataxia and narcosis

Endpoint/Concentration/Rationale: No deaths after 30-min exposure to 7970 ppm followed by a 14-day observation period.

Uncertainty Factors/Rationale:
Total uncertainty factor: 10; AEGL-3 values derived with a larger UF would conflict with AEGL-2 values.
Interspecies: factor of 3 because 1) data were comparable for rats and guinea pigs suggesting no large interspecies differences and 2) the critical effect is CNS depression.
Intraspecies: factor of 3 because interindividual variability for CNS depression by comparable gasses generally will not be greater than a factor of 2 to 3.

Modifying Factor: None

Animal to Human Dosimetric Adjustment: None

Time Scaling: Default values of 3 and 1 to 10-min and 60-min, respectively. AEGL-3 values for 4- and 8-hours are set equal to the 1-hour value because 1) chlorobenzene concentrations in blood reach a steady-state within 1 hour and elimination is rapid and 2) time scaling would result in 4- and 8-hour AEGL-3 values that would conflict with AEGL-2 values and human data.

Data Adequacy: Sufficient.

1
2